JAPANESE PATENT OFFICE (JP)

Official Gazette for Unexamined Patent Applications (A)

(11) Japanese Unexamined Patent Application (Kokai) No. 8-99891

(43) Disclosure Date: 16 April 1996

(51)	Int.Cl.$_6$	Ident. Symbols	Internal Office Nos.	FI	Technology Indication
	A61K 35/78 7/00	ADS S K ADA X	8217-4C		

Request for Examination: Not yet requested Number of Claims: 4 (Total of 7 pages)

(21) Application No.: 6-22228

Application Date: 21 January 1994

Applicant: 000113470
Pola Chemical Industrial Company, Ltd.
6-48 Yayoi-cho, Shizuoka-shi, Shizuoka-ken

Inventor: Akio Fujii
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

Inventor: Yasuji Ogo
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

Inventor: Kenkichi Matsubara
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

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(54) Title of the Invention: A Topical Skin Agent

[Abstract]

[Structure] A melanin production inhibitor that is comprised of essence of seeds of plants of the family Curcurbitaceae and a topical skin agent that contains them.

[Effect] The melanin production inhibitor of this invention is of high safety and superior melanin production inhibiting action. Topical skin agents in which it is compounded have superior beautifying-whitening action.

[Claims]

[Claim 1] A melanin production inhibitor that is comprised of essence of seeds of plants of the family Curcurbitaceae.

[Claim 2] A melanin production inhibitor as described in Claim 1 in which the plant of the family Curcurbitaceae is Benincasa hispida Thummb Cogn..

[Claim 3] A topical skin agent that contains the melanin production inhibitor as described in Claims 1 and 2.

[Claim 4] A topical skin agent as described in Claim 3 in which the content of melanin production inhibitor as described in Claims 1 and 2 is 0.0001 to 10 weight %.

[Detailed Description of the Invention]

[0001]

[Field of industrial use] This invention relates to a melanin production inhibitor and to a topical skin agent that contains it. In greater detail, [this invention relates to] melanin production inhibitors that are of essence of seeds of plants of the family Curcurbitaceae and a topical skin agent that contains them.

[0002]

[Prior art] It is the desire of many women to have white, beautiful skin. Skin that was white during infancy gradually undergoes pigment deposition and loss of beauty over the years or due to the effects of ultraviolet rays. Pigment deposition is thought to occur when melanin is produced as tyrosine is changed to dopa and dopa is changed to dopa quinone by the enzyme tyrosinase, which is biosynthesized in pigment cells and via intermediates such as 5,6-dihydroxyindole.

[0003] From this standpoint, sulfur containing compounds such as glutathione and hydroquinoine derivatives, which have an inhibiting action of tyrosinase activity and hydrogen peroxide solutions, ascorbic acid and derivatives thereof which bleach the melanin that is produced have been developed as substances for inhibiting pigment deposition. However, these substances present problems in terms of safety, decomposition, coloration and generation of unpleasant odors. In addition, there are also not a few problems of safety such as formation of irreversible white spots and occurrence of rashes. Consequently, it is no exaggeration to say that melanin production inhibitors fit for practical use have not yet been developed.

[0004] On the other hand, it is known that seeds of plants of the family Cucurbitaceae have a melanin production inhibiting action.

[0005]

[Problems the invention is intended to solve] This invention was developed in the light of these circumstances. Consequently, it has the objective of providing melanin production inhibitors of superior safety and melanin production inhibition and cosmetic materials that contain them.

[0006]

[Means for solving the problems] On the basis of the viewpoint described above, the inventors collected raw drugs that were thought to be of superior safety and concerning which there were records of use for many years and they conducted repeated screenings with melanin production inhibiting action as the index and perfected this invention by discovering that winter melon seeds, which are seeds of Benincasa hispida Thummb Cogn. of the family Curcurbitaceae have a marked melanin production inhibiting action.

[0007] Specifically, this invention relates to melanin production inhibitors comprised of essence of seeds of plants of the family Curcurbitaceae.

[0008] This invention further relates to topical skin agents that contain melanin production inhibitors comprised of essence of seeds of plants of the family Curcurbitaceae.

[0009] We shall now present a detailed description of this invention. Seeds of plants of the family Curcurbitaceae that are used as the melanin production inhibitors in this invention are widely used for various purposes as a Chinese traditional medicinal drug. For example, trichosanthis seed, which are seeds of Korean crow gourd [Translator's Note: Literally translated from the Japanese. We were not able to reference this material in the various specialized glossaries available to us.], are used for moisturizing the lung and transforming phlegm and for ulcers. Toka seeds, which are seeds of Benincasa hispida Thummb Cogn. are used for clearing the lungs, transforming phlegm, disinhibiting dampness and expelling pus [Translator's Note: These are terms used in traditional Chinese medicine.]. Of the seeds of plants of the family Curcurbitaceae that are used in this invention, the most desirable are tokashi, which are seeds of Benincasa hispida Thummb Cogn.. This is because Benincasa seeds are of superior melanin production inhibiting action and safety.

[0010] The term essence of seeds of plants of the family Curcurbitaceae of this invention refers to seeds of plants of the family Curcurbitaceae in unaltered state, to seeds that have been dried and finely pulverized, to extracts obtained by subjecting the seeds to extraction with a polar solvent and removing the solvent and to fractions obtained by fractionating extracts by column chromatography or fluid separation extraction.

[0011] There are methods for obtaining extracts from seeds of plants of the family Curcurbitaceae. They can be performed by ordinary extraction methods. Before the extraction procedure, it is desirable to perform pre-treatments such as drying and pulverizing the seeds in advance. In these cases, solvent is added to the seeds or pretreated seeds in amounts of 1 to 100 times their volume. They are immersed for several hours as the temperature is being raised or for several days if they are at room temperature and the insoluble matter is removed by filtration, after which they may be dried under reduced pressure and the solvent removed. Solvents of high polarity are particularly desirable. For example, there can be one or two or more solvents selected from water, methanol, ethanol, propanol, butanol, acetone, diethyl ether, chloroform, methylene chloride and dichloroethane.

[0012] The extract that is obtained in this way may be compounded in an unaltered state with the topical skin agent and it may also be compounded after fractionation and purification by column chromatography or liquid-liquid extraction. As specific examples of fractionation and purification we can cite silica gel chromatography using a mixed solution of methanol and diethyl ether or a mixed solution with chloroform as the elution solvent, ODS column chromatography in which a methanol aqueous solution or an acetone aqueous solution as the elution solvent and liquid-liquid extraction with butanol-water, diethyl ether-water, ethyl acetate-water and hexane-water.

[0013] The essence that has been obtained in this way can be made into a topical skin agent preparation with various optional components in accordance with ordinary methods. There are no particular limitations on the form of the preparation as long as it is a form that is ordinarily used such as a lotion, a cream, an ointment an emulsion or a pack. Optional components can include polyvalent alcohols, humectants, thickeners, hydrocarbons, esters, alcohols, higher fatty acids, surfactants, powdered components, colorants, fragrances, antioxidants, ultraviolet ray absorbents and antiinflammatory agents. In addition, other melanin production inhibitors such as sulfur-containing compounds, ascorbic acid derivatives and hydroquinone derivatives may also be compounded.

[0014] The content of melanin production inhibitor of this invention in the topical skin agent should be 0.0001 to 10 weight %. When it is less than 0.0001 weight %, melanin production inhibiting activity cannot be anticipated. When it exceeds 10 weight %, the effect is already at its maximum and the increased amount is not economical. It is preferable that the content of melanin production inhibitor of this invention in the topical skin agent be 0.1 to 10 weight %, at which level the melanin production inhibiting action is at a maximum.

[0015]

[Examples] We shall now describe this invention in greater detail by presenting examples. However, it goes without saying that this invention is not limited by these examples.

[0016] Example 1

Example of Manufacture

1 liter of a 50% aqueous solution of methanol was added to seeds of Benincasa hispida Thummb Cogn., heating and reflux were performed for 3 hours and 7.5 g of melanin production inhibitor 1 was obtained as a pale yellow amorphous substance.

[0017] Example 2

Example of Manufacture

7.5 g of melanin production inhibitor 1 as described above was dispersed in 300 ml of water, 300 ml of normal hexane was added and the mixture was thoroughly agitated, after which solution separation was performed, the normal hexane layer was removed and dried under reduced pressure, with 170 mg of melanin production inhibitor 2 being obtained as a pale yellow amorphous substance. 300 ml of ethyl acetate was added to the remaining aqueous layer and the mixture was thoroughly agitated, after which the ethyl acetate layer was collected and dried under reduced pressure, with 360 mg of melanin production inhibitor 3 being obtained as a pale yellow amorphous substance. 300 ml of normal butanol was added to the remaining aqueous layer and the mixture was thoroughly agitated, after which the butanol layer was collected and dried under reduced pressure, with 850 mg of melanin production inhibitor 4 being obtained as a pale yellow amorphous substance. The remaining aqueous layer was dried under reduced pressure and 5960 mg of melanin production inhibitor 5 was obtained as a pale yellow amorphous substance.

[0018] Example 3

Melanin production inhibiting action

A study was conducted of the melanin production inhibiting action of the melanin production inhibitors 1 to 5 obtained in Examples 1 and 2 using melanoma B-16 cells. Specifically, B-16 cells in the logarithmic growth period were treated with trypsin, a MEM culture medium suspension of 1.5×10^3 cells/ml containing 10% FBS (bovine fetal serum) was prepared and this suspension was poured in amounts of 10 ml each into individual culture bottles. They were cultured for 2 days in a CO_2 incubator (37°C, 5% CO_2). Various concentrations of melanin production inhibitor were added and culturing was continued for 2 days. The culture medium was discarded on the 6th day and the culture was washed with PBS (phosphate buffer physiological saline solution), after which trypsin treatment was performed and the cells were peeled off. Following this, the cells were collected by centrifugation and were observed visually, with evaluations being made for cytotoxicity and melanin production inhibitory action. The criteria for cytotoxicity were as follows. +: cytotoxicity was present; ±: evaluation of cytotoxicity was difficult; -: cytotoxicity was not present. The criteria for melanin production inhibitory action were as follows: +: melanin production inhibitory action was present; ±: very slight melanin production inhibitory action was found; -: melanin production inhibitory action was not present. The results are shown in Table 1. It was found that the melanin production inhibitors of this invention inhibited the production of melanin satisfactorily even at low concentrations.

[0019]

[Table 1]

Samples	Concentration (%)	Melanin production inhibition	Cytotoxicity
Melanin production inhibitor 1	0.1		
Melanin production inhibitor 2	0.004 0.002 0.0002	+ + ±	± - -
Melanin production inhibitor 3	0.01 0.005	+ ±	+ -
Melanin production inhibitor 4	0.02	±	-
Melanin production inhibitor 5	0.17	±	±

[0020] Example 4

Safety (local toxicity)

In order to ascertain the safety of the melanin production inhibitors of this invention, a local toxicity study was conducted by percutaneous injection using Hartley white guinea pigs (males, 350 g). Specifically, 1% ethanol solutions of melanin production inhibitors 1 to 5 were administered 5 times a day in doses of 0.05 ml each into 2 cm square sites that were made by shaving the backs of the guinea pigs. On the 6th day, the skin reaction was evaluated on the basis of the Japanese Patch Test Criteria (Japanese Dermatology Society); specifically. -: no reaction; ±: false positive reaction; +: positive reaction; ++: reaction accompanied by edema. The results were negative reactions (-) in all cases. Thus, it was found that the melanin production inhibitor of this invention exhibited excellent safety.

[0021] Example 5

Example of Compounding (Toilet Water)

Toilet water was prepared in accordance with the formulation indicated below. Specifically, the components indicated below were weighed out, dissolved by heating at 80°C and cooled, with toilet water being obtained.

Propylene glycol	5.5
Ethanol	10
Methylparaben	0.2
Sodium chloride	0.3
Citric acid	0.1
Sodium acetate	0.1
Fragrances	0.1
Polyoxyethylene (50) hardened castor oil	0.5
Water	83.1
Melanin production inhibitor 1	0.1

[0022] Example 6

Example of Compounding (Toilet Water)

Toilet water was prepared in accordance with the formulation indicated below. Specifically, the components indicated below were weighed out, dissolved by heating at 80°C and cooled, with toilet water being obtained.

Propylene glycol	5.5
Ethanol	10
Methylparaben	0.2
Sodium chloride	0.3
Citric acid	0.1
Sodium acetate	0.1
Fragrances	0.1
Polyoxyethylene (50) hardened castor oil	0.5
Water	82.2
Melanin production inhibitor 2	1

[0023] Example 7

Example of Compounding (Toilet Water)

Toilet water was prepared in accordance with the formulation indicated below. Specifically, the components indicated below were weighed out, dissolved by heating at 80°C and cooled, with toilet water being obtained.

Propylene glycol	5.5
Ethanol	10
Methylparaben	0.2
Sodium chloride	0.3
Citric acid	0.1
Sodium acetate	0.1
Fragrances	0.1
Polyoxyethylene (50) hardened castor oil	0.5
Water	73.2
Melanin production inhibitor 1	10

Toilet water was prepared in accordance with the formulation indicated below. Specifically, the components indicated below were weighed out, dissolved by heating at 80°C and cooled, with toilet water being obtained.

[0024] Example 8

Propylene glycol	5.5
Ethanol	10
Methylparaben	0.2
Sodium chloride	0.3
Citric acid	0.1
Sodium acetate	0.1
Fragrances	0.1
Polyoxyethylene (50) hardened castor oil	0.5
Water	83.1
Melanin production inhibitor 3	0.01

[0025] Example 9

Example of Compounding (Cream)

A cream was prepared in accordance with the formulation indicated below. Specifically, A and B were weighed out separately, dissolved by heating at 80°C, B was gradually added to A as the materials were being stirred and emulsification was effected. The product was cooled as the materials were being stirred, with a cream being obtained.

(A)	Cetanol	1
	Synthetic spermaceti	2.5
	Beeswax	2.5
	stearic acid	1
	Vaseline	3
	Squalane	14
	Olive oil	6
	γ-Tocopherol	0.1
	Fragrances	0.1
	Butylparaben	0.1
	Glyceryl monostearate	2.5
	Polyoxyethylene (25) stearate	2.5
(B)	Sodium hydroxide	0.02
	Melanin production inhibitor 2	0.03
	Water	56.4
	Propylene glycol	8
	Methylparaben	0.25

[0025] Example 10

Example of Compounding (Cream)

A cream was prepared in accordance with the formulation indicated below. Specifically, A and B were weighed out separately, dissolved by heating at 80°C, B was gradually added to A as the materials were being stirred and emulsification was effected. The product was cooled as the materials were being stirred, with a cream being obtained.

(A)	Cetanol	1
	Synthetic spermaceti	2.5
	Beeswax	2.5
	stearic acid	1
	Vaseline	3
	Squalane	14
	Olive oil	6
	γ-Tocopherol	0.1
	Fragrances	0.1
	Butylparaben	0.1
	Glyceryl monostearate	2.5
	Polyoxyethylene (25) stearate	2.5

(B)	Sodium hydroxide	0.02
	Melanin production inhibitor 1	0.0002
	Water	56.4298
	Propylene glycol	8
	Methylparaben	0.25

[0026] Example 11

Example of Compounding (Cream)

A cream was prepared in accordance with the formulation indicated below. Specifically, A and B were weighed out separately, dissolved by heating at 80°C, B was gradually added to A as the materials were being stirred and emulsification was effected. The product was cooled as the materials were being stirred, with a cream being obtained.

(A)	Cetanol	1
	Synthetic spermaceti	2.5
	Beeswax	2.5
	stearic acid`	1
	Vaseline	3
	Squalane	14
	Olive oil	6
	γ-Tocopherol	0.1
	Fragrances	0.1
	Butylparaben	0.1
	Glyceryl monostearate	2.5
	Polyoxyethylene (25) stearate	2.5
(B)	Sodium hydroxide	0.02
	Melanin production inhibitor 5	0.3
	Water	56.13
	Propylene glycol	8
	Methylparaben	0.25

[0027] Example 12

Example of Compounding (Cream)

A cream was prepared in accordance with the formulation indicated below. Specifically, A and B were weighed out separately, dissolved by heating at 80°C, B was gradually added to A as the materials were being stirred and emulsification was effected. The product was cooled as the materials were being stirred, with a cream being obtained.

(A)	Cetanol	1
	Synthetic spermaceti	2.5
	Beeswax	2.5
	stearic acid`	1
	Vaseline	3
	Squalane	14
	Olive oil	6
	γ-Tocopherol	0.1
	Fragrances	0.1
	Butylparaben	0.1
	Glyceryl monostearate	2.5
	Polyoxyethylene (25) stearate	2.5

Sodium hydroxide	0.02
Melanin production inhibitor 1	0.003
Water	56.427
Propylene glycol	8
Methylparaben	0.25

[0028] Example 13

Example of Compounding (Foundation)

A foundation was prepared in accordance with the formulation indicated below. Specifically, A was mixed by kneading, after which B was added and the materials were further mixed by kneading. The materials were heated to 80°C, after which C was dispersed and D, which had been dissolved by heating at 80°C was gradually added and emulsification was effected. The materials were stirred and cooled, with a foundation being obtained.

Propylene glycol	5
Malvitol [phonetic]*	10
Methylparaben	0.3
Glycerol triisostearate	4
Liquid paraffin	5
Butylparaben	0.1
Titanium oxide	9
Yellow iron oxide	1.7
Red iron oxide	1.2
Talc	8.1
Water	55
Melanin production inhibitor 5	0.6

[0029]

[Effect of the invention] The melanin production inhibitors of this invention exhibit excellent safety, and, moreover, their melanin production inhibiting activity is also high, for which reason they are very advantageous. Consequently, topical skin agents that contain them are also safe and have superior melanin production inhibiting action and are therefore very advantageous.

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(72) Inventor: Takeshi Hara
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

(72) Inventor: Satoru Obata
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

*Translator's Note: Transliterated from the Japanese. As such, the spelling may differ from other transliterations.

Inventor: Takuji Nakajima
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

Inventor: Yoshiyuki Miyata
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken

Inventor: Yoshio Kitada
c/o Yokohama Research Alboratories
Pola Chemical Industrial Company, Ltd.
27-1 Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa-ken